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Sex Reversal and Growth Performances in *Clarias gariepinus* (Burchell, 1822) Fry Fed Dietary Genistein under Laboratory Conditions

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ABSTRACT

Monosex fish stocks are desirable in aquaculture in order to control reproduction and select the gender that displays faster growth characteristics. The present study investigated the sex reversal potential and growth performance of *Clarias gariepinus* fry fed dietary genistein, extracted from soybean meal. Four experimental diets (A, B, C, and D) were formulated at 40% crude protei; diets (C and D) supplemented with genistein at 0.05g/100g diet along with control diet were fed to *C. gariepinus* fry for twenty eight days. Higher number of males were recorded in the fry fed diet containing both genistein and soybean meal but vice versa in the case of diet containing genistein without soybeans. Soybean diet supplemented with genistein could be used to reverse sex in the fish hatchery for sustainable aquaculture.

KEY WORDS: Aquaculture, Sex Reversal, Phytochemical, Genistein, *Clarias gariepinus*

INTRODUCTION

In order to reduce the pressure on the wild stocks of fish, aquaculture is a viable alternative to fisheries throughout the world [1]. Manipulation of phenotypic sex in fish farming is generally desirable since one gender, depending on the fish species, grows faster than the other [2]. Over the years, aquaculturists have engaged the use of exogenous sex steroids such as 17 α-Methytestoterone (MT) to accomplish complete sex-reversal in fish culture production [3, 4]. However, the latent health effect and environmental hazards make the use of alternative chemicals for sex-reversal in aquaculture to be explored [5]. One promising alternative approach may involve the use of natural plant extracts such as isoflavonids, flavonoids and saponins as they possess aromatase inhibitory ability to suppress estrogen biosynthesis in cells [6], and are ecofriendly. Akinwande *et al*. (2011) [6] stated that genistein (*3,5,7,3,4 pentahydroxyflavone*) induced increased level of endogenous testosterone as indication of aromatase inhibition in rainbow trout.

In view of the forgoing, the present study was carried out on *Clarias gariepinus* fry in other to investigate sex reversal potential and evaluate growth performance of dietary genistein.

MATERIALS AND METHOD

The study was conducted in the Laboratory of the Department of Fisheries, Modibbo Adama University of Technology, Yola, Nigeria.

Genistein Extraction

The genistein used in the present study was extracted from toasted soybean flour, using Enhance Solvent Extraction method of AACC as described by Dowing *et al*. (1999) [7].

Spawning of Experimental Fish

A total of 2 male and 4 female brooders were used for spawning in cylindrical plastic tanks of 75L capacity. 2 weeks old fry were collected after hatching from the plastic tank using a hapa sampling net and the fry were gently sorted out.

Experimental Design/Diet

Fry were randomly distributed into 25L plastic tanks with the water level maintained at 15L and tanks were continuously aerated. The fish were stocked at 20 fry per tank. Initial mean weight of fry per tank was taken using electronic top – loading balance (Mettler, E200).

Four diets (A, B, C, and D) were formulated as shown in Table 1. Fish were fed the experimental diets two times daily at 5% body weight for 28 days. The fish were kept under 12 hours light and 12 hours darkness. Weekly weights were monitored for growth determination and quantity of feed fed were adjusted accordingly. The experimental fish were also monitored for mortality.

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Sex Ratio/Growth Performance Evaluation

Sexes were determined through urogenital papilla for male and genital papilla for female. Growth performance was evaluated in terms of growth parameters including specific growth rate (%/day), weight gain (g) and relative growth rate (%).

Statistical Analysis

Data generated from the experiment was subject to analysis of variance (ANOVA) using Graphpad Insta window 7.

RESULTS AND DISCUSSION

Table 2 presents proximate values of the four experimental diets; diets A, B, C, and D indicated crude protein of 43.88%, 42.69%, 43.00%, and 41.19% respectively and were not significantly different ($p>0.05$). The growth performance as presented in Table 3 showed that genistein inclusion in the diets has no significant positive effect on the growth response. Table 4 documented the sex ratio of *C. gariepinus* fed each of the four experimental diets.

Fish fed diets C and D had lower mean weight gain; relative growth rate and specific growth rate as compared to diets A and B. The lower growth responses recorded by both diets (C and D) suggested that the presence of experimental (extracted) "genistein" in the diets at 0.05g/100g diet might be above the growth inducing level for the fish. Francis *et al.* (2001) [8] reported increase in weight in Nile tilapia fed the experimental diets containing saponins from soybeans at a concentration of 300mg/kg of feed. Moreover, the lowest mean weight gain; relative growth rate and specific growth rate recorded by diet D might have been complemented by the additional genistein from soybean meal used in the diet formulation; as genistein is also present in soybean meal [9]. Ko *et al.* (1999) [10] in their report on the effect of genistein on the growth and reproductive development of yellow perch, *Perca flavescens*, indicated that the genistein diet at a concentration of 7.5 mg g^{-1} diet decreased weight gain in females, while the growth of the fish fed diet containing genistein at a concentration of 0.75 mg g^{-1} diet showed no difference in growth. However, the results of Turan and Akyurt (2005) [11] from an experiment to evaluate the effects of a red clover, *Trifolium pretense*, extract, containing high levels of genistein, on growth and body composition of *C. gariepinus* indicated that the fish fed diets containing red clover extract at concentrations of 75 mg kg^{-1} diet showed the highest final weight, weight gain, specific growth rate.

Diet D which contained experimental (extracted) "genistein" and soybeans had the highest number of males. This might be as a result of the combined effects of the experimental "genistein" with the phytochemicals in soybeans. The report of Makkar *et al.* (2007) [12] indicated that phytochemicals have bioactive influence on sex in aquaculture and livestock products and that other phytochemicals especially saponin, a major phytochemical in soybeans, influences masculinization in fish especially as recorded in Tilapia. Ghosal and Chakraborty, (2014) [13] reported significant increase in percentage of males in *Oreochromis niloticus* treated with aqueous leaf extract of *Basella alba*, which was reported to show presence of phytochemicals such as tannins, saponins, steroids and alkaloids. Also, increased proportions of male and intersex individuals were observed in Channel catfish fed diets containing genistein at 4 and 8 mg g^{-1} concentration between 5 and 140 days post-hatch [9]. These authors reported that this sex reversal might have resulted from the dual role of genistein as not only an oestrogen agonist but also as an antagonist blocking oestrogen's action.

Based on the results and forgoing, it could be concluded that genistein aromatase inhibitory property might be more potent if combine with other phytochemicals especially those of soybeans. However, it is suggested that in further studies, dietary inclusion levels of genistein should be varied and in combination with other phytochemicals, probably the sex ratio might be dosage/combination dependent.

Table 1: Composition of the Experimental Diets

Ingredients	Diet A (Control)	Diet B	Diet C	Diet D
Maize	20.0	20.0	20.0	20.0
Fish meal	38.0	38.0	38.0	38.0
Soybeans	-	18.0	-	17.0
GNC	36.0	18.0	35.95	18.95
Genistein	-	-	0.05	0.05
Starch	2.0	2.0	2.0	2.0
Palm oil	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5
Bone meal	0.5	0.5	0.5	0.5
Vitamin premixes	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0

Table 2. Proximate Composition of Experimental Feeds

Feed sample	Protein (%)	Lipids (%)
Diet A	43.88	2.46
Diet B	42.69	8.56
Diet C	43.00	7.40
Diet D	41.19	7.78

Table 3. Growth and Feed Utilization of *C. gariepinus* Fry Fed Experimental Diets

Indices	Diet A	Diet B	Diet C	Diet D
Mean initial weight (g/fish)	0.26^{b}	0.24^{b}	0.32^{a}	0.38^{a}
Mean final weight (g/fish)	2.52	2.66	2.50	2.48
Mean weight gain (g/fish)	2.26^{ab}	2.42^{a}	2.18^{b}	2.10^{b}
Feed intake	0.44^{a}	0.39^{b}	0.43^{ab}	0.45^{a}
Feed conversion ratio	0.20^{b}	0.15^{a}	0.20^{b}	0.21^{b}
Protein intake (mg/100g of diet)	19.22^{a}	16.78^{b}	18.28^{a}	18.70^{a}
Protein efficiency rate	0.13^{b}	0.16^{a}	0.14^{b}	0.13^{c}
Relative growth rate (%)	869.0^{b}	1008.3^{a}	681.35^{c}	552.6^{d}
Specific growth rate (%/day)	3.52^{a}	3.73^{a}	3.19^{b}	2.91^{c}

Data with different superscripts are significantly different ($p<0.05$)

Table 4. Sex Ratio of *C. gariepinus* Fry Experimental Diets

Feed Sample	Total No. of fish Examined	Females	Males
Diet A	18	11	7
Diet B	18	13	5
Diet C	15	12	3
Diet D	19	6	13

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